## APPENDIX A: EQUIPMENT INVENTORY

The following list includes all materials, solutions and equipment needed to run *Ceriodaphnia* toxicity tests using the SIMPLIFIED PROTOCOL. The list is divided by potential sources of these items.

### 1.0 Toxicity Testing Kit (to be supplied to teachers etc. by a government agency)

Disposable equipment and materials:

one syringe without needle, 5 ml, for feeding
one syringe without needle, 60 ml
one syringe without needle, 5 ml, for KCl
plastic pipette with built in bulb, 5 ml, cut at the end
cerio cups, 24 per test of 6 treatments plus 3 for temperature measurements (1 oz Solo plastic cups without lids)
stock solution 10 g/l potassium chloride (KCl) in distilled water
"Alconox" detergent for jar cleaning, a few grams for the entire project

Equipment on loan:

one 100 micron sieve
one 400 micron sieve
one small flat cup (2 or 3 oz Solo plastic cup without lid)
one conductivity meter
one pH meter or a pack of non-bleeding pH strips
Minimum-maximum thermometer

### 2.0 Test organisms and food (To be supplied by local toxicity laboratories)

*Ceriodaphnia* culture starter (40 organisms or more)
YCT mixture, 120 ml per month
*Selenastrum* concentrate, 120 ml per month

Participating toxicity laboratories:

Pacific Eco-Risk, Martinez (Scott Ogle, (510) 313-8080)
Block Environmental Services, Pleasant Hill (Ron Block, (510) 682-7200)
MEC, Tiburon (Paul Krause, (415) 435-1847)
ToxScan, Watsonville (Dave Lewis, (408) 724-4522)

### 3.0 Local grocery (to be purchased by user)

Arrowhead Spring Water, 1 gallon per month
Evian mineral water, 1 liter per month
Distilled/purified water, supermarket grade
Disposable 9 oz clear plastic cups, 3 or more per test

## 4.0 Classroom and home (Attic/garage/kitchen) items

Clear, wide mouth glass jars without lids for *Ceriodaphnia* cultures, 800-1000 ml

Clear, wide mouth glass jars with lids lined with inert material (or an empty sandwich bag), 250 or 500 ml, for samples

Permanent marking pen

Cerio board (bottoms of egg cartons, or a Styrofoam board with holes for cerio cups)

Flat flashlight under wire shelf or frame, and opaque white surface on top, for a Light Table (if needed)

Hand lens (or a microscope) to observe dead and living *Ceriodaphnia*

Small bulb thermometer

## 5.0 Other suppliers

If your school can spend $25-35 a month for *Ceriodaphnia* food, you may include supplier-information in your protocol. A company called Aquatic Biosystems Inc. (1-800-331-5916), located in Colorado, will ship *Ceriodaphnia* food by UPS overnight service (COD possible). In 1994 they charged $15 for 0.5 liter of *Selenastrum* food suspension and $10 for 0.5 liter of frozen YCT mixture; these quantities should suffice for 3-4 weeks.

The 400 micron net is commercially available under the trademark Nitex, which is a nylon netting. It may be purchased, for example, under Catalog # E-NT-NTX 400 from Argent Chemical Laboratories, 8702 157th Avenue North East, Redmond, WA 98052 (Telephone number 800-426-6258). Similarly, the 100 micron net is sold under catalog # E-NT-NTX-100. Pieces of one square meter are the minimum size sold, and it costs approximately $40.

## APPENDIX B: QUALITY ASSURANCE GUIDELINES

This section identifies how accurate, precise, complete, comparable, sensitive, well documented, valid, and representative our toxicity testing and measurements will be, and what we can do to make them even better. In some cases we do not have enough information to provide a good evaluation (e.g., for representativeness or accuracy), due to constraints of our sampling design and to the nature of toxicity tests. However, if we are aware of these limitations, uncertainties, and sources of error, we can qualify the results so that any potential user will know how reliable our data are. Quality assurance/quality control (QA/QC) plans include several "elements" that are formally applied to assure and control the quality of data. The following guidelines discuss the applicability and utility of these QA/QC elements to our protocol.

### B-1. GENERAL QA/QC CONSIDERATIONS

#### Record keeping / Chain of custody / Completeness

Use the data sheets provided throughout the protocol, as you work, and be sure to include all the information that the protocol is asking for. These sheets are also your reporting format, so they must be legible.

- Form TTS30*: CERIODAPHNIA* CULTURE LOG - to be filled daily by the culture maintenance crew
- Form TTS35: FIELD DATA SHEET FOR TOXICITY STUDY SAMPLING - to be filled by each sampler, for each sample, at the time of sampling. These forms are also your "chain of custody" records.
- DATA SHEET FOR *CERIODAPHNIA* TOXICITY TEST Page 1: Control and Reftox, and Page 2: Samples - to be filled when the test is set up and on every observation during and after the test.

It is assumed that every procedure of the test is performed according to the protocol, so there is no need to record these details again. However, if you add something or do something different, keep clear records of any additions to or deviations from the protocol.

**Holding time** : Generally, samples for toxicity testing should be refrigerated and tested as soon as possible or within 36 hours of collection from a constant discharge. However, if the samples were collected during a rain event or a temporary dry weather discharge, they should be tested within 72 hours.

**Lack of Contamination:** Sampling containers and laboratory utensils need to be carefully cleaned to assure that no toxicity, apart from the toxic substances potentially present in the sample (for which we are testing the sample), is inadvertently introduced.

**Representativeness:** This element is about how well the sample we have collected represents the environment that we have sampled, both in the temporal sense (e.g., what flows in the creek or street gutter during the entire storm event) and in the spatial sense (e.g., what flows in the same gutter at the same time 100 yards from where we are). The sampling suggestions provided in this protocol allow for a high degree of uncertainty about representativeness. However, if several samples are collected from a given environment, and pooled together, this can increase the representativeness.

## B-2. QA/QC ELEMENTS SPECIFIC TO TOXICITY TESTING

Beyond the quality assurance/quality control (QA/QC) elements of sample collection, custody, and handling, QA plans for toxicity testing are designed to show that test procedures were adhered to, including water quality measurements, and that clear records are kept of any deviations from the protocol. These plans have procedures and criteria to show that all the test organisms were healthy and properly fed, that the control organisms survived and reproduced adequately, and that the organisms exposed to reference toxicants were not too sensitive or too resistant.

### Test Validation Criteria:

1. Control survival should be at least 80%.

2. Reference toxicant tests are used to establish a laboratory's ability to obtain precise results, and also to establish an acceptable range of sensitivities of test organisms. This range is established for each combination of reference toxicant and test organism. For this protocol, potassium chloride (KCl) has been selected as a reference toxicant. The salt is used at two concentrations, reftox 1 (a concentration that is not expected to kill Ceriodaphnia more than 95% of the time) and reftox 2, a concentration that is expected to kill the organisms more than 95% of the time. The test validation criteria are defined as: at least 80% survival in reftox 1 (150 mg/l potassium chloride), and 50% survival or less in reftox 2 (500 mg/l potassium chloride). As of June 1998, the San Francisco Bay Area test results with these concentrations using adult Ceriodaphnia at room temperature has not been compiled to generate relevant statistics and confidence intervals.

### Water Quality

Commercial laboratories testing environmental samples with *Ceriodaphnia* also has to show that the test organisms had enough oxygen, that the correct test temperature was maintained, that the pH values were not extreme, and that the organisms were not subject to osmotic stress. Water chemistry parameters are monitored daily during the test to ensure that the animals are exposed to environmental conditions which will not cause a "toxic effect" by

themselves. The values should fall within the ranges known as "safe" for the organisms, and the laboratory is instructed to modify some parameters of the sample before exposure to prevent extreme ("out of range") conditions if necessary.

However, this simplified protocol for science students does not recommend modification of a sample before it is used for the test, and calls for measurements of pH and electrical conductivity (EC) at test initiation and termination only. In fact, this protocol calls for measurements of pH and EC so we can tell if mortality could be "explained" by environmental conditions that were not "safe", rather than change the conditions. The "safe", or "physiologically comfortable" conditions are: pH in the range of 6.5-8.8, and conductivity of 40-3000 microsiemens (μS). Temperatures in the range of 10-28 degrees Celsius (perhaps even lower temperatures) can be tolerated, but the test is conducted at room temperature rather than at the temperature at which the sample was collected. However, remember that any measurement outside these ranges does not invalidate the test.

**Precision and Accuracy**

The precision of a toxicity test is an expression of the degree of reproducibility of results, and it can be determined by evaluating the variability among laboratory replicates and by analyzing duplicate samples. Although this element has not been formally incorporated into the present protocol, variability among the four replicates can be evaluated, and duplicate samples may be analyzed from time to time.

Accuracy is the nearness of a measurement to its true value. In a biological toxicity test, accuracy is enhanced by using several replicate chambers for each sample. However, the "true value" of toxicity cannot be determined. This is because toxicity is a relative rather than an absolute concept, since only organisms can "measure" toxicity, and there is no true or absolute reference organism. Toxicity test results (e.g., percent survival) can be compared to each other, but their deviation from a true value cannot be determined. This is different from chemical quantification, in which standard analyte solutions or buffers are used to establish the true concentration or to calibrate instruments (see below).

**Consistency / Comparability**

Many times it is difficult to determine if a test organism is really dead, and different observers may have different "signs". The protocol provides a criterion to help make a decision (animal on bottom and does not move even after gentle tapping or swirling of the cup); this little "test" should be done when there is doubt. Consistent use of the same criteria, procedures, and data sheets by all programs will ensure that "everybody is on the same page", and will allow comparisons of the data. There is an inherent source of

uncertainty about various sensitivities of test organisms from different laboratories. However, the use of reference toxicants provide a means of reducing uncertainties associated with organism sensitivity.

## B-3. WATER QUALITY MEASUREMENTS

The precision of temperature or pH measurements can be formally evaluated by recording measurements of the same containers by different team members, measurements of different replicates of the same sample at the end of the test, etc. Duplicate samples are also useful, to account for the influence of sample jars. The degree of reproducibility of data is often expressed as "Relative Percent Difference" (RPD) which is the difference between the two readings, divided by the average of the two, and multiplied by one hundred. For example, if the conductivity readings of two duplicate samples were 180 and 220 microsiemens (μS), the RPD is (220-180)/200X100=20%.

To assure accuracy, instrument calibration for pH is recommended before each day-use, and for conductivity one time during the project. Thermometer readings should be compared to the best mercury thermometer available. In all measurements, the operator should be familiar with the time needed for equilibration or stabilization of the readings and wait until stabilization before recording the value.

The following table specifies the Data Quality Objectives recommended for Water Quality measurements:

| Parameter | Method/range | Units | Detection Limit | Sensitivity | Precision (RPD) | Accuracy |
|---|---|---|---|---|---|---|
| Temperature | Thermometer (0 - 50°C) | °C | NA | 1.0 °C | 20% | ± 1.0 |
| pH | pH meter (3-12) | pH units | 3 | 0.1 unit | 10 % | ± 0.3 |
| | pH strip (non-bleed, 5-12) | pH units | 5 | 0.5 unit | NA | ± 0.5 |
| Conductivity | Conductivity meter (10-1990) | umhos/cm | 10 | 10 umhos/cm | 20% | ± 20 |

NA - not applicable
RPD - Relative Percent Difference - is the difference between the two readings, divided by the average of the two, and multiplied by one hundred.